

random. Domains were directly observed. The domain size depended on the type of the membrane anchor (HRas: 130 nm, KRas: 200 nm; NRas: 200 nm). Further we found that domain formation and disassembly was dynamic on a timescale of 5-30s.

#### 1204-Plat

##### Super-Resolution Localization Microscopy Identifies Distinct Stages of Antigen-Induced IgE Receptor Cross-Linking and Immobilization in Rbl-2H3 Mast Cells

Sarah A. Shelby<sup>1</sup>, David A. Holowka<sup>2</sup>, Barbara A. Baird<sup>2</sup>, Sarah L. Veatch<sup>3</sup>.

<sup>1</sup>Field of Biophysics, Cornell University, Ithaca, NY, USA, <sup>2</sup>Chemistry and Chemical Biology, Cornell University, Ithaca, NY, USA, <sup>3</sup>Biophysics, University of Michigan, Ann Arbor, MI, USA.

Cross-linking of immunoglobulin E (IgE) bound to its receptor, FcεRI, by multivalent antigen initiates a transmembrane signaling cascade essential for mast cell activation and important for inflammatory immune responses and allergic disease. In this study, we apply super-resolution fluorescence localization microscopy to record receptor organization and dynamics on live RBL-2H3 mast cells undergoing antigen-mediated signaling, allowing us to measure nanoscale clustering and diffusion of FcεRI simultaneously. Through comparison of cross-linking-induced changes in these properties as a function of time, we are able to resolve two distinct temporal phases of receptor clustering and immobilization. Additionally, we correlate the time-dependence of the distinct phases with a functional signaling response, Ca<sup>2+</sup> mobilization. In the first phase of receptor clustering and immobilization, receptors slow dramatically with a relatively small average increase in clustering, and individual receptors appear to transiently associate with small clusters. This first phase occurs before Ca<sup>2+</sup> mobilization and concurrently with initial signaling steps. At later times, receptor-rich clusters become increasingly dense while receptors remain predominately immobilized. These latter behaviors are observed at times following the initiation of the Ca<sup>2+</sup> response, and we conclude that although cross-linking is necessary for commencement of downstream signaling, receptor assembly into large, densely packed clusters at later times is likely associated with termination of the stimulated response. These findings motivate future study of the physical interactions that give rise to the observed changes in receptor organization and mobility, and how these translate into cellular functions. In ongoing experiments, we are exploring the requirements of signaling for receptor cross-linking through the use of antigens with controlled structure and valency, and we will correlate our observations with functional responses such as Ca<sup>2+</sup> mobilization and receptor association with downstream signaling partners.

#### 1205-Plat

##### The Actin Cytoskeleton Controls the Activation of Invariant Natural Killer T Cells by Fine-Tuning CD1d Nanoscale Aggregation on Antigen Presenting Cells

Juan Andres Torreno-Pina<sup>a</sup>, Michael Aichinger<sup>b</sup>, Carlo Manzo<sup>a</sup>, Mariolina Salio<sup>b</sup>, Vincenzo Cerundolo<sup>b</sup>, Maria F. Garcia-Parajo<sup>a,c</sup>.

<sup>a</sup>ICFO, Institut de Ciències Fotòniques, Mediterranean Technology Park, 08860 Castelldefels, Barcelona, Spain, <sup>b</sup>MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK, <sup>c</sup>ICREA, Institutació Catalana de Recerca i Estudis Avançats, 08010, Barcelona.

Invariant Natural killer T (iNKT) cells are a subset of lipid-specific T cells, restricted by the MHC-I class like molecule CD1d. A recent study showed a correlation between the co-localization of lipid-loaded CD1d molecules with lipid rafts on the membrane of antigen presenting cells, and their capacity of eliciting secretion of Th1-cytokines by stimulated iNKT cells [1]. Thus, this study suggested that not only the structure of CD1d bound to an exogenous lipid could influence CD1d-mediated immunity, but also its partitioning on the membrane. Here, we address the spatiotemporal behaviour of α-Galactosylceramide loaded CD1d complexes on the cell membrane of human myeloid cells using multiple colour high-speed single-particle tracking (100 Hz) combined with an iNKT T Cell Receptor-Qdot conjugate as imaging probe. Furthermore, we complement these studies using STED *nanoscopy* to obtain nanoscale images of CD1d spatial organization. Our results indicate a direct role of the actin cytoskeleton in actively segregating CD1d nanoclusters on the cell membrane resulting in an inhibition of the activation of iNKT cells [2]. As a whole, our work proposes a new paradigm of *biophysical* interaction between CD1d presenting cells and NKT cells which deviates significantly from classical MHCII complexes and CD8/CD4-T Cells interactions.

[1] J. S. Im et al., *Immunity*, **30**, 888, 2009.

[2] J. A. Torreno-Pina et al., in preparation.

#### 1206-Plat

##### Multi-Color, Single-Molecule Fluorescence Imaging of GPCR Signalosomes

Thomas Huber<sup>1</sup>, Alexandre Fürstenberg<sup>2</sup>, He Tian<sup>1</sup>, Hubert F. Gaertner<sup>2</sup>, Oliver Hartley<sup>2</sup>, Thomas P. Sakmar<sup>1</sup>.

<sup>1</sup>Laboratory of Chemical Biology & Signal Transduction, www.sakmarlab.org, Rockefeller University, New York, NY, USA,

<sup>2</sup>Department of Pathology and Immunology, University of Geneva, Geneva, Switzerland.

G protein-coupled receptors (GPCRs) are the largest superfamily of membrane receptors in the human genome and they are targets for a quarter of all prescription drugs. Activation of a GPCR by an agonist ligand results in G protein-mediated downstream signaling, followed by kinase action and arrestin-mediated desensitization, internalization/sequestration, and recycling. Selective manipulation of these individual steps of the GPCR activation cycle is often desired when creating drugs targeting a given receptor. We are interested in the C-C chemokine receptor CCR5 that is the major HIV coreceptor used in person-to-person transmission. Globally, the HIV/AIDS pandemic has caused nearly 30 million deaths and a similar number of people are currently infected. Certain analogues of the chemokine RANTES/CCL5 are highly potent entry inhibitors against R5-tropic HIV-1 strains, in vitro and in vivo. Three such analogues, 5P12-, 5P14-, and 6P4-RANTES, are particularly interesting because while they differ only slightly in structure they show strikingly different pharmacological profiles (G protein-linked signaling activity, stimulation of receptor internalization). We have recently developed a general, simple, and robust method for stoichiometric, site-specific fluorescence labeling of expressed GPCRs. The method is based on bioorthogonal conjugation of a fluorescent reporter group to a genetically encoded azido group introduced into expressed GPCRs using amber codon suppression.[1] We have adopted a similar strategy for fluorescent labeling of chemokines with azido groups introduced by chemical synthesis. Here we present our progress towards automated, multi-color, single-molecule fluorescence studies of the compositional and conformational dynamics of GPCR signaling complexes ("signalosomes") using fluorescently labeled chemokines and receptors in biochemically defined systems.

[1] H Tian, TP Sakmar, & T Huber (2013) Site-specific labeling of genetically encoded azido groups for multi-color, single-molecule fluorescence imaging of GPCRs. *Methods in Cell Biology*, 117, in press.

#### 1207-Plat

##### Dengue Virus Infection Mediated by DC-SIGN

Ping Liu, Marc R. Ridilla, Aravinda M. de Silva, Nancy L. Thompson, Ken Jacobson.

UNC at Chapel Hill, Chapel Hill, NC, USA.

DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin) is a pattern recognition receptor which binds to the mannose or fucose structures present on a variety of pathogens and stimulates diverse immune responses. DC-SIGN forms nanodomains on cell surfaces which are entry portals for viruses including HIV, Ebola, dengue and hepatitis C. In particular, dengue is a mosquito-borne viral infection and has become a rapidly growing global health threat. Many reports have shown that ectopically expressed DC-SIGN enhances productive dengue infection in different human cell types; however, detailed molecular-level studies on interactions between DC-SIGN membrane assemblies and dengue virus (DENV) at the initial binding and internalization stages are lacking. By employing immunostaining, confocal imaging, super-resolution direct stochastic optical reconstruction microscopy (dSTORM) and flow cytometry assays, we show that cell surface DC-SIGN nanodomains are sufficient to capture the small sized DENV (50 nm), leading to efficient virus internalization and productive infection of the host cells. At the initial binding stage, DENV is highly colocalized with cell surface DC-SIGN domains. Internalization of DENV was observed within a few minutes after incubating DENV with cells expressing DC-SIGN, and massive viral particle synthesis was observed at 24h after infection. In contrast, no virus replication was observed on control cells even after 72h of incubating with DENV. The results indicate that DC-SIGN capturing of DENV leads to rapid internalization of the viruses and productive infection thereafter. Furthermore, superresolution dSTORM shows that a single DC-SIGN nanodomain is sufficient to capture single DENV particles. Supported by NIH GM 41402 and NIAID RO1-A1107731.

#### 1208-Plat

##### How Talin Head Domain and Soluble Ligand Contribute to Integrin αIIbβ3 Activation

Mehrdad Mehrbod, Stephen Trisno, Mohammad R.K. Mofrad.

Bioengineering, University of California, Berkeley, Berkeley, CA, USA.

Integrin αIIbβ3 is widely known to regulate the process of thrombosis via activation at its cytoplasmic side by talin and interacting with soluble fibrinogen. Three groups of interactions regulate integrin activation: a set of salt bridges